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Automatic Fuzzy Inference System development for marker-based watershed segmentation

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Abstract. Texture image segmentation is a constant challenge in digital image processing. The partition of an image into regions that allow the experienced observer to obtain the necessary information can be done using a Mathematical Morphology tool called the Watershed Transform. This transform is able to distinguish extremely complex objects and is easily adaptable to various kinds of images. The success of the Watershed Transform depends essentially on the existence of unequivocal markers for each of the objects of interest. The standard methods for marker detection are highly specific and complex when objects presenting great variability of shape, size and texture are processed. This paper proposes the automatic generation of a fuzzy inference system for marker detection using object selection done by the expert. This method allows applying the Watershed Transform to biomedical images with diferent kinds of texture. The results allow concluding that the method proposed is an effective tool for the application of the Watershed Transform.

1. Introduction
Biomedical images are formed by objects presenting a high variability in shapes, sizes and intensities. They are highly textured and have a high level of noise, low contrast and great spatial resolution.

The partition of an image into regions that allow the experienced observer to obtain the necessary information can be done using a Mathematical Morphology tool called the Watershed Transform (WT). This transform is able to distinguish extremely complex objects and is easily adaptable to various kinds of images.

The WT is a segmentation method based on regions, which classifies pixels according to their spatial proximity, the gradient of their gray levels and the homogeneity of their textures. To avoid over-segmentation, a single marker for each object of interest has to be selected.

The selection of adequate markers on these kinds of images is a hard and sometimes fruitless task. Hence, the experienced observer usually resorts to defining markers in a semiautomatic way [1][2]. The automatic determination of markers still is a difficult goal to achieve. Current determination algorithms are highly dependent on the structure to be segmented [3][4]. Moreover, they have a high computational cost and they determine markers in an effective but not automatic way when processing highly textured images [5]-[7].

This paper proposes the automatic generation of a fuzzy inference system for the detection of markers by means of object selection done by the expert.

2. Materials
For this work, microscope images were used in order to evaluate the proposed algorithms due to the great difficulty that their segmentation presents. Bone marrow biopsies were used in order to evaluate the proposed algorithms. In these images, the trabeculae need to be segmented in order to make a

diagnosis. A dye with hematocylin and eosyn was applied to bone marrow tissue to color label the trabeculae. Although the original biopsies are in color, we turned them into grey levels to facilitate their processing. Image adquisition of the samples were made with an optic microscope Medicux-12 and a CCD camera Hitachi KP-C550. Image resolution is 640 x 480 pixels, and they were saved in Windows bitmap (BMP). Even though this image is formed by different biological structures, those of the trabeculae are the most difficult ones to segment. For this reason they were used to evaluate the algorithms. The images were acquired through a microscope where light, resolution and contrast were set by the lab technician to obtain the best visualization possible.

In order to segment these images, a histogram cannot be used, because grey levels characteristic of the trabeculae cannot be distinguished as well [6][10]. Due to the great variability of these conditions, it is impossible to define markers automatically with the methods developed so far.

All the algorithms were implemented in Matlab® R14. We worked with the standard functions of this language and a specific library called SDC Morphology Toolbox (SDC, 2001) with functions of mathematical morphology.

3. Methods

3.1. Watershed Transform (WT)

WT is a segmentation method based on regions that classifies pixels according to their spatial proximity, the gradient of their gray levels and the homogeneity of their textures [7], [12]-[14].

A grey scale image can be interpreted as the topographic image of land relief. It can be indicated that the gray intensities of higher amplitude correspond to plains and mountains and the lower intensity ones correspond to valleys and rivers [15]-[16]. Using the characteristics of these images we define a technique for digital image processing called WT which, by means of flooding the valleys, is capable of recognizing similar topographical areas, surrounded by mountain ridges.

With the objective of segmenting an image in gray levels, prior to the application of the WT, a gradient image must be obtained, where the contour levels of objects to be segmented represent an area of higher grey intensity. The areas of low intensity give way to the basins where the water would flow and flood the topography of the image. The elevations in gray levels generated by the contours would remain and give way to the segmentation of the image through the resulting watershed lines. Mathematical morphology allows us to obtain a gradient which is highly adaptable to different kinds of images, with higher precision than conventional algorithms. In this paper we used the morphological gradient to obtain the intermediate image before applying the WT [11]-[14].

The classic WT floods the gradient image from its regional minima. In non-homogeneous or noise embedded images there is not a one-to-one relationship between regional minima and objects of interest. This usually results in over-segmentation of images; i.e., after WT each of the objects is represented by more than one region [5]. To avoid this over-segmentation we resort to choose a single marker for each object of interest. These markers -or seeds- initiate the flooding algorithms by indicating the sector that gives rise to the basins [6]-[9]. On the grounds of these characteristics, we can conclude that the success of the WT depends mainly upon the characteristics of the markers.

3.2. Fuzzy Inference System

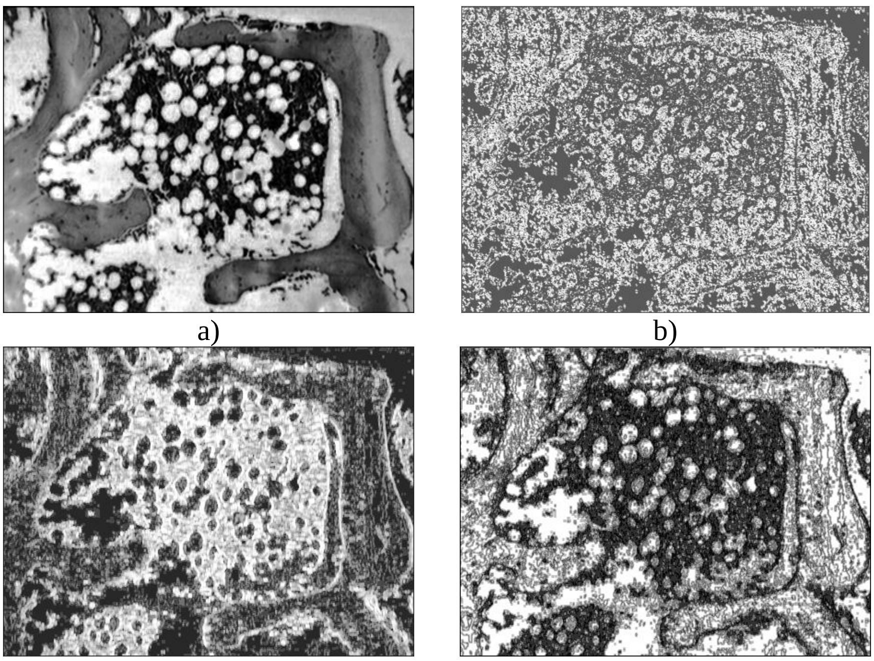
In this paper, we propose to use fuzzy logic as a tool to assist in the determination of markers. This discipline arises from the formalization of imprecise, ambiguous and linguistically expressed knowledge [9]-[11]. There is an ambiguity inherent to image processing that can be found, for example, in topological images that project a 3D relief onto a 2D image; in the digitalization of microscope images, where resolution, definition and contrast depend on the user and the applications in particular; in contours obtained with low definition; in images of textured and non homogeneous regions; and in medical images, where the figure is not only a photograph, but also the quantification of a certain kind of energy of stochastic nature that interacts with the body resulting in blurred and imprecise images.

A fuzzy inference system processes information from input variables to give output values. It uses the values of input variables to determine the truth values of the predicates that it will use as antecedents in a rule base. Each value of the input variables is assigned a value of membership to different fuzzy sets defined for each variable. This procedure is done through membership functions which can be, for instance, trapezoid or Gaussian functions. Through an inference process for each rule (determination of consequents) and a following process of aggregation (union of results from the different rules), we obtain values for the output variables for different values of the input variables.

Nowadays there are segmentation methods that apply fuzzy logic to the entropy function of the histogram of an image, with the objective of improving and segmenting it. They define fuzzy membership functions through the image histogram to discriminate objects or regions. However, when it comes to textured images, it is not posible to differentiate objects in an automatic manner by only using the histograms of grey intensity levels or its attributes [9]-[11].

3.3. Proposed Algorithm
The proposed method consists in relating the characteristics of the different components of an image based on its texture. A fuzzy inference system is generated (Mamdani Fuzzy Inference System) from the indication of the object of interest that is to be segmented by the experienced observer. Finally the WT is applied to the gradient image of the original image from the obtained markers.

The texture characterization of image components was made with co-ocurrence matrices [14]-[16]. The co-ocurrence matrices were built from masks that analyse the grey-level distribution of the contours of each pixel. Contrast, maximum value and energy were calculated. Masks of 3x3 size were used (figure 1). Masks of a larger size (5x5, 7x7, 10x10 and 15x15) reduced the definition of object contours, thus preventing their use. Other characteristics were also analysed, e.g. the mean grey value of each contour and the gradient. The values of characteristics that were analysed belong into the interval [0,1]. They were moved to the interval [min, max] = [0,255] to normalize them and to obtain values possible to be visualized in a gray scale image. Normalization also enabled the chosen parameters to become independent from image lighting.



a) b)

c) d)

Figure 1. Texture characteristics obtained with a 3x3 mask.
a) Mean value. Characteristics determined from the co-ocurrence matrix: b) energy c) contrast, d) maximum value.

The next step consisted in obtaining with the aid of an experienced observer two points of the image to be segmented. The fist one corresponded to a point belonging to the object of interest; in this case, the trabeculae. The second point belonged to the background of the image.

The values of characteristics for each point were later used to define the membership functions of the fuzzy inference system [8]-[10]. The proposed system has the objective of attenuating the background and irrelevant objects and making objects of interest stand out.

First, we determined the mean value of the characteristics found in a 9x9 space surrounding the points selected by the observer. Then, we generated the Gaussian membership functions, whose highest membership points corresponded to mean values obtained in the first step. This was done for each characteristic; not only for the background but also for the trabeculae, for each specific image.

A rule base was determined from these membership functions, considering the information the experienced observer provided by distinguishing the background from the trabeculae. The output of the inference system has two membership functions: the first one, to indicate the presence of the object and the second, the background. This inference system was used for each pixel of the image using the values of characteristics corresponding to each píxel.

Some rules used for the three texture characteristics are:

If the píxel has an energy value that corresponds to the energy of the object of interest, then it is object.

If the píxel has an energy value that corresponds to the energy of the background, then it is background.

If the píxel has a contrast value corresponding to the contrast of the object of interest, then it is object.

If the píxel has a contrast value that corresponds to the background contrast, then it is background.

If the píxel has a mean value that corresponds to the mean value of the object of interest, then it is object.

If the píxel has a mean value that corresponds to the mean value of the background, then it is background.

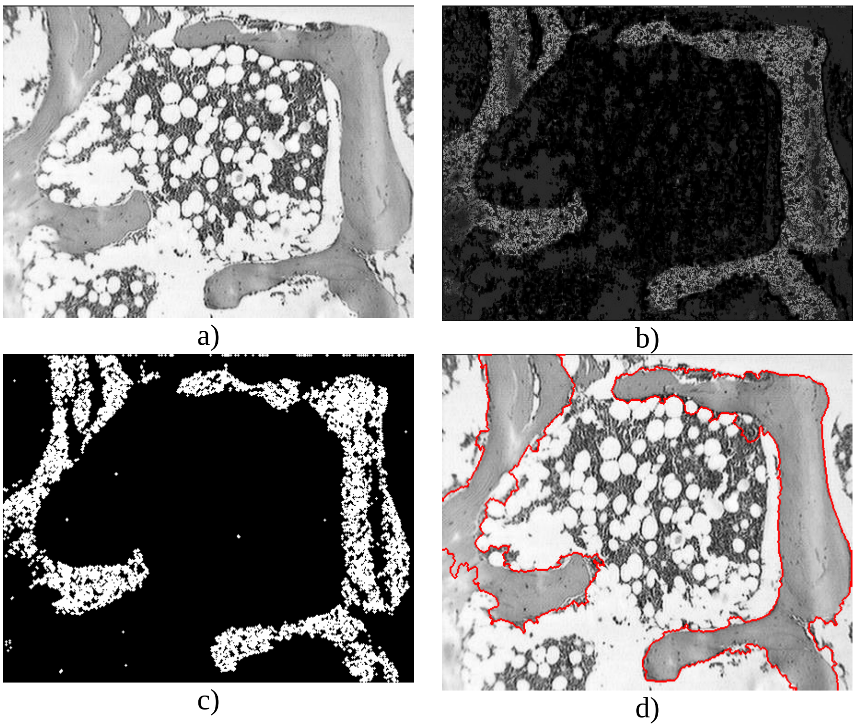


Figure 2. a) Bone marrow biopsy; b) Result using the fuzzy system; c) Binarization; d) Result using WT.

The proposed rule base enabled to obtain high levels of gray when the pixel corresponded to the object, and low levels of gray when it corresponded with the background (figure 2-a, 2-b).

A conventional binarization with a threshold in 128 allowed to differentiate the objects (figure 2-c) [15]-[16]. However, we observed grainy texture in the objects of interest that prevented to use this result to define markers for the WT. Therefore, new openings with structuring elements of 3x3 pixels was applied, obtaining unequivocal and homogeneous internal markers for each object of interest [16].

In order to apply the WT, it is necessary to mark not only the objects but also the background. Not only do we need to define the internal markers for the objects of interest but also the external markers. The latter ones were obtained through the morphological erosion of the complement of the internal markers.

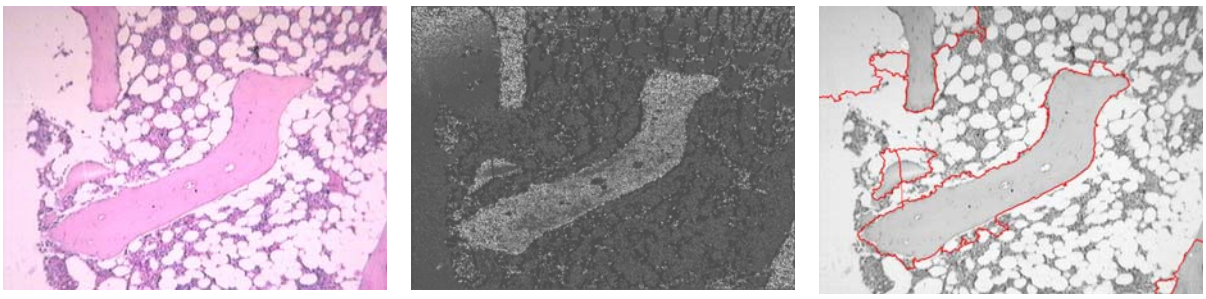
This procedure resulted in adequate segmentations when we obtain internal markers of a similar size to those of the object of interest. These properties of the markers were attained using the proposed algorithm.

Finally, the WT was applied to the gradient image of the original image using the markers obtained in the previous steps (figure 2-d) [5]-[7]. It was not possible to obtain this result with other conventional methods of image segmentation.

4. Results

Figure 3 shows the images resulting from the application of the proposed algorithm, where the successful segmentation of the trabeculae can be seen. It was possible to analyse biopsies from various patients presenting different pathologies, and that could not be segmented with other algorithms for marker detection. Twenty-four images belonging to different regions of biopsies from eight different patients were processed in total. On the other hand, results evaluation showed that the process is robust; that is, it is not dependent upon the pixel selected by the operator, as long as such points lie internally to the objects, rather than on their contours. This is so because the texture of contours differs from that of the interior of the object, resulting in values of texture characteristics differing significantly one another.

The performance of the segmentation algorithm was evaluated on other images. It was applied to images captured with a microscope, because of their high texture level. They corresponded to samples of bacteria and lymph nodes. The same inference system applied to bone marrow biopsies was applied to the images, and this allowed defining the internal markers for each object of interest. Figure 4 shows the correct segmentation of the objects of interest by WT using said markers.



a) b) c)

Figure 3. a) Bone marrow biopsy. b) Result with fuzzy inference system.
c) Result from applying WT.

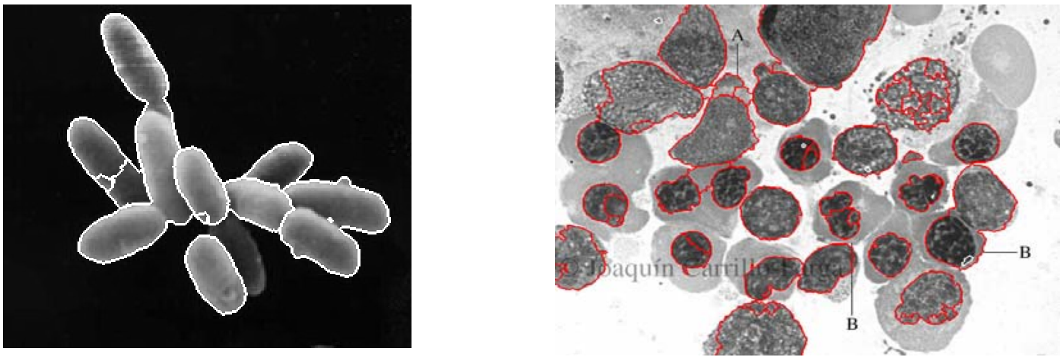


Figure 4. Result after applying the proposed algorithm: Left-side frame: halobacteria; right-side frame: lymph nodes

5. Conclusions

The use of fuzzy logic to define markers for WT is an adequate means because it is not necessary to obtain the complete object, but only an approximation of its interior. The fuzzy inference system generated from having an experienced observer select the object of interest allows for a correct definition of internal markers, and a consequent successful application of the WT. The main advantage of the inference system developed here is that the determination of the markers through mathematical morphology, after applying the rule base, is simple and robust. This is not posible to do with the original image without previously applying the fuzzy inference system to the texture characteristics.

Trabeculae corresponding to biopsies of different patients and pathologies were successfully segmented. The proposed method is flexible enough as to be applicable to all kinds of image. These features make the system adaptative to any particular image without needing to modify either the algorithm or its parameters.

6. References

[1] Glasbey C A and Horgan G W 1994 *Image análisis for the biological science* ed Vic Barnett, Jonh Wiley and Sons (Statistics in Practice, Series)

[2] Gauch J M 1999 Image segmentation and analisys via multiescale gradient watershed hierarchies *IEEE Trans. on Image Processing* **8**:1

[3] Li P and and Xiao X 2004 An unsupervised marker image generation metod for watershed segmentation of multiespectral imagery *Geoscience Journal* **8** 325-31

[4] Pratikakis I E, Sahli H and Cornelis J 1999 Low level image partitioning guided by the gradient watershed hierarchy *Signal Processing* **75** 173-95

[5] Stanislav L S and Straber W 2000 Extracting regions of interest appying a local watershed transformation, *Proc. of IEEE Visualization*

[6] Gonzalez M A and Ballarin V L 2006 Segmentación de imágenes mediante la Transformada Watershed: Obtención de marcadores mediante Morfología Matemática *9vo Simposio Argentino de Informática y Salud (SIS*), *Universidad Nacional de Cuyo*

[7] Vincent L and Soille P 1991 *Watersheds in digital spaces : An efficent algorithm based on immertion simulations* IEEE trans. Pattern Anal (Machine Intell.) vol. 13 pp. 583-98

[8] Wang D 1997 A multiscale gradient algorithm for Image Segmentation using Watersheds, *Pattern Reconition* **30** 2043-52

[9] Ceccarelli M, Musacchia F and Petrosino A 2006 A fuzzy scale-space approach to feature-based image representation and retrieval *Brain Vision and Artificial Intelligence* 377-385

[10] Ceccarelli M and Petrosino A 2002 A paralel fuzzy scale-space approach to the unsupervised texture separation *Pattern Recognition Letters* 23 **5**: 557-567
[11] Cheng H D, Chen Y H and Sun Y 1999 A novel fuzzy entropy aproach to image enhancement and thresholding *Signal Processing* **75** 277-301
[12] Beucher S and Meyer F 1993 *The morphological approach to segmentation: The Watershed Transformation, Mathematical Morphology and its aplication to image processing* ed Marcel Decker (New York) chapter 12 pp. 433-81
[13] Beucher F 1994 Topografic distance and watershed lines *Proce. Inst. Workshop on MM ans its application to signal processing* 113-25
[14] Couprie M and Bertrant G 1997 Topological grayscale Watershed Transform, *SPIE Vision Geometry Proceedings* **3168** 136-46
[15] Castleman K R 1979 *Digital Image Processing* (Prentice Hall) chapter 15
[16] Dougherty E R and Astola J 1994 *An introduction to nonlinear image prossesing* **TT16** (Tutorial Texts in optical engineering)